Rec'd PCT/PTO 04 JUN 2001

FORM PTO-1390 (Rev. 1-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY 'S DOCKET NUMBER JAB-1430

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
09/857372

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| PCT/EP99/09833 | December 6, 1999 | December 4, 1998 |

TITLE OF INVENTION
DRUG TARGETS IN CANDIDA ALBICANS

APPLICANT(S) FOR DO/EO/US :
CONTRERAS

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).

9. ☒ An executed oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☒ Assignment documents (3) for recording. Separate cover sheets in compliance with 37 CFR 3.28 and 3.31 are included.

13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☒ A substitute specification in 71 pages including 30 claims, a sequence listing, and 4 figures.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: Copy of the International Preliminary Examination Report; Copy of the International Search Report; Associate Power of Attorney; Sequence Disk; Sequence Listing, Verified Statement.

JC18 Rec'd PCT/PTO 04 JUN 2001

| U.S. APPLICATION NO. (if known, see 37 CFR 1.5) | INTERNATIONAL APPLICATION NO. | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| 09/857372 | PCT/EP99/09833 | JAB-1430 |

17. ☒ The following fees are submitted: — CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO................ $1070.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO...........$930.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.455(a)(2)) paid to USPTO...... $790.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)....... $720.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)............. $98.00

| | | | | | |
|---|---|---|---|---|---|
| ENTER APPROPRIATE BASIC FEE AMOUNT = | | | | $ 930.00 | |
| Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)). | | | | $ | |
| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | |
| Total claims | 30 - 20 = | 10 | x $18.00 | $180.00 | |
| Independent claims | 20 - 3 = | 17 | x $80.00 | $1360.00 | |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | | + $270.00 | $ | |
| TOTAL OF ABOVE CALCULATIONS = | | | | $2470.00 | |
| Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). + | | | | $ | |
| SUBTOTAL = | | | | $ | |
| Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | $ | |
| TOTAL NATIONAL FEE = | | | | $2470.00 | |
| Fee for recording the enclosed 3 assignments (37 CFR 1.21(h)). The assignments must be accompanied by an appropriate cover sheets (37 CFR 3.28, 3.31). $40.00 per property + | | | | $120.00 | |
| TOTAL FEES ENCLOSED = | | | | $2470.00 | |
| | | | | Amount to be refunded: | $ |
| | | | | charged: | $ 2590.00 |

a. ☐ A check in the amount of $________ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 10-0750/JAB-1430/MHM in the amount of $2270.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 10-0750/JAB-1430/MHM. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

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June 4, 2001

09/857372 JC18 Rec'd PCT/PTO 04 JUN 2001
JAB-1430

# DRUG TARGETS IN *CANDIDA ALBICANS*

## Field of the Invention

The present invention is concerned with the identification of genes or functional fragments thereof from *Candida albicans* which are critical for growth and cell division and which genes may be used as selective drug targets to treat *Candida albicans* associated infections. Novel nucleic acid sequences from *Candida albicans* are also provided and which encode the polypeptides, which are critical for growth of *Candida albicans*.

## Background of the Invention

Opportunistic infections in immunocompromised hosts represent an increasingly common cause of mortality and morbidity. *Candida* species are among the most commonly identified fungal pathogens associated with such opportunistic infections, with *Candida albicans* being the most common species. Such fungal infections are thus problematical in, for example, AIDS populations in addition to normal healthy women where *Candida albicans* yeasts represent the most common cause of vulvovaginitis.

Although compounds do exist for treating such disorders, such as, amphotericin, these drugs are generally limited in their treatment because of their toxicity and side effects. Therefore, there exists a need for new compounds, which may be used to treat *Candida* associated infections in addition to compounds, which are selective in their action against *Candida albicans*.

Classical approaches for identifying anti-fungal compounds have relied almost exclusively on inhibition of fungal or yeast growth as an endpoint. Libraries of natural products, semi-synthetic, or synthetic

chemicals are screened for their ability to kill or arrest growth of the target pathogen or a related nonpathogenic model organism. These tests are cumbersome and provide no information about a compounds mechanism of action. The promising lead compounds that emerge from such screens must then be tested for possible host-toxicity and detailed mechanism of action studies must subsequently be conducted to identify the affected molecular target.

**Summary of the Invention**

The present inventors have now identified a range of nucleic acid sequences from *Candida albicans*, which encode polypeptides, which are critical for its survival and growth. These sequences represent novel targets which can be incorporated into an assay to selectively identify compounds capable of inhibiting expression of such polypeptides and their potential use in alleviating diseases or conditions associated with *Candida albicans* infection.

In a first aspect of the invention, the invention relates to a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9. More preferably where nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 3 and still more preferably consisting of SEQ ID NO:1 and SEQ ID NO:2.

In a preferred embodiment of this aspect of the invention, the nucleic acid molecule is either RNA or DNA, and in one embodiment, cDNA. The invention also relates to a first nucleic acid molecule capable of hybridising to a second nucleic acid molecule encoding a polypeptide which is critical for survival and

growth of the yeast *Candida albicans* and which second nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 under high stringency conditions. In yet another embodiment, the invention relates to an antisense molecule comprising a first nucleic acid molecule capable of hybridising to a second nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* wherein the second nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

The invention also relates to cells containing a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein said cells are bacterial or eukaryotic. The invention further relates to polypeptide encoded by a nucleic acid molecule which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 and to polypeptide having any of amino acid sequences selected from the group consisting of SEQ ID NO: 10 to SEQ ID NO: 16.

The invention also relates to a recombinant DNA construct comprising a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein the nucleic acid molecule is DNA. Preferably the DNA is an expression vector and preferably the expression vector includes an inducible promoter and/or a reporter molecule. The invention also relates to recombinant DNA constructs comprising a first nucleic acid molecule wherein the first nucleic acid molecule or a molecule

complementary to the first nucleic acid molecule is capable of hybridising to a second nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which second nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 wherein the first nucleic acid molecule is inserted in the antisense orientation.

The invention also relates to cells containing a recombinant DNA construct comprising a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein said cells are bacterial or eukaryotic.

In another aspect of this invention, the nucleic acid molecules or polypeptides of this invention are provided in a pharmaceutically acceptable carrier, diluent or excipient.

The invention further relates to a method for treating a *Candida albicans*-associated disease comprising the step of: administering a composition of matter comprising an antisense nucleic acid molecule capable of binding to a *Candida albicans*-originating nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9.

In another aspect of this invention, the invention relates to a *Candida albicans* cell comprising an induced mutation in the DNA sequence encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* wherein the polypeptide is encoded by a nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

The invention also relates to a method of identifying compounds which selectively modulate expression or functionality of polypeptides or

metabolic pathways in which these polypeptides are involved and which polypeptides are crucial for growth and survival of *Candida albicans*, which method comprises: contacting a compound to be tested with one or more *Candida albicans* cells having a mutation in a nucleic acid molecule, the nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 wherein the mutation results in overexpression or underexpression of said polypeptide in addition to contacting one or more wild type *Candida albicans* cells with said compound; and monitoring the growth and/or activity of said mutated cell compared to said wild type; wherein differential growth or activity of said one or more mutated *Candida* cells is indicative of selective action of said compound on a polypeptide or another polypeptide in the same or a parallel pathway. The invention further relates to compounds identified by this method. The compounds can be provided in a pharmaceutical composition with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The invention also relates to a method of identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are critical for growth or survival of said cell or organism, which method comprises: preparing a cDNA or genomic library from said cell or organism in a suitable expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription of antisense RNA from the nucleotide sequences in said cDNA or genomic library; selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant. In a preferred aspect

of this invention, the cell or organism is a yeast or filamentous fungus and more preferably the cell or organism is any of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

The invention further relates to antibody capable of specifically binding to a polypeptide of this invention. The invention also relates to oligonucleotides comprising a fragment of from 10 to 120 contiguous nucleotides of a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9. Preferably the oligonucleotide comprises a fragment of from 10 to 50 contiguous nucleotides.

**Brief Description of the Figures**

Figure 1 is a diagrammatic representation of plasmid pGAL1PNiST-1.

Figure 2 is a diagrammatic representation of plasmid pGAL1PSiST-1.

Figure 3 is an exemplary growth curve of a Candida albicans strain demonstrating antisense-induced reduction in growth in response to intracellular antisense expressing specifically targeting one of the nucleic acid sequences of this invention.

Figure 4 is a representative growth curve of a *Candida albicans* knock out strain as compared to wild type *Candida albicans*.

**Detailed Description of the Invention**

According to a first aspect of the invention there is provided a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid

molecule comprises any of the sequences of nucleotides illustrated in any of Sequence ID Nos. 1 to 9.

Whilst the molecules defined herein have been established as being critical for growth and metabolism of Candida albicans, for some of the molecules no apparent functionality has been assigned by virtue of the fact that no functionally related sequences in other prokaryotic or eukaryotic organism can be found in respective databases. Thus, advantageously these sequences may be species specific in which case they may be used be used as selective targets for treatment of diseases mediated by *Candida Albicans* infection. Thus, in one aspect of the invention the nucleic acid molecules preferably comprise the sequences identified in sequence ID Nos. 1, 4, 5 to 9.

In another aspect of the invention the sequences have been arranged functionally and of nucleotides illustrated in Sequence ID Nos. 2 or 3 are preferred and even more preferably in Sequence ID No. 2 and fragments or derivatives of said nucleic acid molecules.

Letters utilised in the sequences according to the invention which are not recognisable as letters of the genetic code signify a position in the nucleic acid sequence where one or more of bases A, G, C or T can occupy the nucleotide position. Representative letters used to identify the range of bases which can be used are as follows:

M: A or C
R: A or G
W: A or T
S: C or G
Y: C or T
K: G or T
V: A or C or G

H: A or C or T

D: A or G or T

B: C or G or T

N: G or A or T or C

In one embodiment of each of the above-identified aspects of the invention the nucleic acid may comprise a mRNA molecule or alternatively a DNA and preferably a cDNA molecule.

Also provided by the present invention is a nucleic acid molecule capable of hybridising to the nucleic acid molecules illustrated in any of Figures 1 to 9 under high stringency conditions such as antisense molecule and which conditions are generally known to those of skill in the art.

Stringency of hybridisation as used herein refers to conditions under which polynucleic acids are stable. The stability of hybrids is reflected in the melting temperature (Tm) of the hybrids. Tm can be approximated by the formula:

$$81.5°C+16.6(Log_{10}[Na^{+}]+0.41\ (\%G\&C)-600L/L$$

wherein L is the length of the hybrids in nucleotides. Tm decreases approximately by 1-1.5°C with every 1% decrease in sequence homology.

The term "stringency" refers to the hybridisation conditions wherein a single-stranded nucleic acid joins with a complementary strand when the purine or pyramidine bases therein pair with their corresponding base by hydrogen bonding. High stringency conditions favour homologous base pairing whereas low stringency conditions disfavour non-homologous base pairing.

"Low stringency" conditions comprise, for example, a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or,

alternatively, a temperature of about 50°C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

"High stringency" conditions comprise, for example, a temperature of about 42°C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65°C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. *et al.* Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

"SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM $Na_2HPO_4$ and 1 mM EDTA, pH 7.4.

The nucleic acid capable of hybridising to nucleic acid molecules according to the invention will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the nucleotide sequences illustrated in any of Figures 1 to 9.

The DNA molecules according to the invention may, advantageously, be included in a suitable expression vector to express polypeptides encoded therefrom in a suitable host which are critical for growth and survival of *Candida albicans*.

An expression vector according to the invention includes a vector having a nucleic acid according to the invention operably linked to regulatory sequences, such as promoter regions, that are capable of effecting expression of said DNA fragments. The term

"operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host cell to provide for expression of a polypeptide according to the invention. Thus, in a further aspect, the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell, transformed, transfected or infected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of said nucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable markers, such as, for example, ampicillin resistance.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for translation initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art.

Polynucleotides according to the invention may be inserted into the vectors described in an antisense

orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense nucleic acids may be produced by synthetic means.

In accordance with the present invention, a defined nucleic acid includes not only the identical nucleic acid but also any minor base variations including in particular, substitutions in bases which result in a synonymous codon (a different codon specifying the same amino acid residue) due to the degenerate code. The term "nucleic acid sequence" also includes the complementary sequence to any single stranded sequence given regarding base variations.

The present invention also comprises within its scope proteins or polypeptides expressed by the nucleic acid molecules according to the invention or a functional equivalent, derivative or bioprecursor thereof.

The present invention also advantageously provides nucleic acid sequences of at least approximately 10 contiguous nucleotides of a nucleic acid according to the invention and preferably from 10 to approximately 120 nucleotides. In another aspect of the invention, nucleotide acid sequences are provided from 10 to 50 nucleotides. These sequences may, advantageously be used as probes or primers to initiate replication, or the like. Such nucleic acid sequences may be produced according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or the like for detecting the presence of a nucleic acid according to the invention. These tests generally comprise contacting the probe with the sample under hybridising conditions and detecting for the presence of any duplex or triplex formation between the probe and any nucleic acid in the sample.

According to the present invention, these probes may be anchored to a solid support. Preferably, they

are present on an array so that multiple probes can simultaneously hybridise to a single biological sample. The probes can be spotted onto the array or synthesized *in situ* on the array. See Lockhart *et al.*, Nature Biotechnology, Vol. 14, December 1996, "Expression monitoring by hybridization to high-density oligonucleotide arrays". A single array can contain more than up to more than a million different probes in discrete locations.

Advantageously, the nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be between approximately 10 to 120 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA from a cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified region or fragment and recovering the amplified DNA. Generally, such techniques as defined herein are well known in the art, such as described in Sambrook *et al* (Molecular Cloning: a Laboratory Manual, 1989).

The nucleic acids or oligonucleotides according to the invention may carry a revealing label. Suitable labels include radioisotopes such as $^{32}P$ or $^{39}S$, enzyme labels or other protein labels such as biotin or fluorescent markers. Such labels may be added to the nucleic acids or oligonucleotides of the invention and may be detected using known techniques *per se*.

The polypeptide or protein according to the invention includes all possible amino acid variants encoded by the nucleic acid molecule according to the invention including a polypeptide encoded by said molecule and having conservative amino acid changes.

Polypeptides according to the invention further include variants of such sequences, including naturally occurring allelic variants which are substantially homologous to said polypeptides. In this context, substantial homology is regarded as a sequence which has at least 70%, preferably 80 or 90% amino acid homology with the polypeptides encoded by the nucleic acid molecules according to the invention.

Nucleic acids and polypeptides which are particularly preferred are those comprising the sequences of nucleotides provided in SEQ ID NOS: 1-3 and polypeptides provided in SEQ ID NOS: 10-12. However, a particularly preferred nucleic acid comprises the sequences of nucleotides provided as SEQ ID NOS: 2 and/or 3, and their corresponding amino acid sequences identified as SEQ ID NOS: 11 and 12.

With reference to the nucleic acids of this invention and the protein encoded thereby, amino acid sequence SEQ ID NO: 10 is the translation of SEQ ID NO:1; SEQ ID NO:1 is the translation of SEQ ID NO:2; SEQ ID NO:12 is the translation of SEQ ID NO:3; SEQ ID NO: 16 is the translation of SEQ ID NO:4; SEQ ID NO:13 is the translation of SEQ ID NO:5; SEQ ID NO:14 is the translation of SEQ ID NO:6 and SEQ ID NO:15 is the translation of SEQ ID NO:7.

Nucleotide sequences according to the invention are particularly advantageous as selective therapeutic targets for treating *Candida albicans* associated infections. For example, an antisense nucleic acid capable of binding to the nucleic acid sequence illustrated in any of SEQ ID NOS: 1-9 may be used to selectively inhibit expression of the corresponding polypeptides, leading to impaired growth of the *Candida albicans* with reductions of associated illnesses or diseases (see Figure 5).

The nucleic acid molecule or the polypeptide according to the invention may be used as a

medicament, or in the preparation of a medicament, for treating diseases or conditions associated with *Candida albicans* infection.

Advantageously, the nucleic acid molecule or the polypeptide according to the invention may be provided in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The present invention is further directed to inhibiting expression of nucleic acids according to the invention *in vivo* by the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation of antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5′ coding portion or the mature protein sequence, which encodes for the protein of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 50 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple-helix - see Lee *et al.* Nucl. Acids Res., 6:3073 (1979); Cooney *et al.*, Science, 241:456 (1988); and Dervan *et al.*, Science, 251: 1360 (1991), thereby preventing transcription and the production of the corresponding protein. The antisense RNA oligonucleotide hybridises to the mRNA *in vivo* and blocks translation of an mRNA molecule into the corresponding protein (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

Antibodies to the protein or polypeptide of the present invention may, advantageously, be prepared by techniques which are known in the art. For example, polyclonal antibodies may be prepared by inoculating a host animal, such as a mouse, with the polypeptide

according to the invention or an epitope thereof and recovering immune serum. Monoclonal antibodies may be prepared according to known techniques such as described by Kohler R. and Milstein C., Nature (1975) 256, 495-497.

Antibodies according to the invention may also be used in a method of detecting for the presence of a polypeptide according to the invention, which method comprises reacting the antibody with a sample and identifying any protein bound to said antibody. A kit may also be provided for performing said method which comprises an antibody according to the invention and means for reacting the antibody with said sample.

Proteins which interact with the polypeptide of the invention may be identified by investigating protein-protein interactions using the two-hybrid vector system first proposed by Chien *et al.* (1991).

This technique is based on functional reconstitution *in vivo* of a transcription factor which activates a reporter gene. More particularly the technique comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain, expressing in the host cell a first hybrid DNA sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or said activating domain of the transcription factor, expressing in the host at least one second hybrid DNA sequence, such as a library or the like, encoding putative binding proteins to be investigated together with the DNA binding or activating domain of the transcription factor which is not incorporated in the first fusion; detecting any binding of the proteins to be investigated with a protein according to the invention by detecting for the presence of any reporter gene

product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein.

An example of such a technique utilises the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate domain for binding to activators upstream of the galactose metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide residues encoding the DNA binding domain of GAL4. These binding domain residues may be fused to a known protein encoding sequence, such as for example the nucleic acids according to the invention. The other vector comprises the residues encoding the protein binding domain of GAL4. These residues are fused to residues encoding a test protein. Any interaction between polypeptides encoded by the nucleic acid according to the invention and the protein to be tested leads to transcriptional activation of a reporter molecule in a GAL4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as ß-galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes.

Further provided by the present invention is one or more *Candida albicans* cells comprising an induced mutation in the DNA sequence encoding the polypeptide according to the invention.

A further aspect of the invention provides a method of identifying compounds which selectively inhibit or interfere with the expression, the functionality of polypeptides expressed from the nucleotides sequences illustrated in any of SEQ ID NOS: 1-9, or the metabolic pathways in which these polypeptides are involved and which are critical for growth and survival of *Candida albicans*, which method comprises (a) contacting a compound to be tested with

one or more *Candida albicans* cells having a mutation in a nucleic acid molecule according to the invention which mutation results in overexpression or underexpression of said polypeptides in addition to one or more wild type *Candida* cells, (b) monitoring the growth and/or activity of said mutated cell compared to said wild type wherein differential growth or activity of said one or more mutated *Candida* cells provides an indication of selective action of said compound on said polypeptide or another polypeptide in the same or a parallel pathway.

Compounds identifiable or identified using the method according to the invention, may advantageously be used as a medicament, or in the preparation of a medicament to treat diseases or conditions associated with *Candida albicans* infection. These compounds may also advantageously be included in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

A further aspect of the invention provides a method of identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are critical for growth or survival, which method comprises (a) preparing a cDNA or genomic library from said cell or organism in a suitable expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription of antisense RNA from the nucleotide sequences in said cDNA or genomic library, (b) selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant. Preferably, the cell or organism may be any yeast or filamentous fungus, such as, for example, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

A further aspect of the invention provides a

pharmaceutical composition comprising any of a compound, an antisense molecule or an antibody according to the invention together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The antisense molecules or indeed the compounds identified as agonists or antagonists of the nucleic acids or polypeptides according to the invention may be used in the form of a pharmaceutical composition, which may be prepared according to procedures well known in the art. Preferred compositions include a pharmaceutically acceptable vehicle or diluent or excipient, such as for example, a physiological saline solution. Other pharmaceutically acceptable carriers including other non-toxic salts, sterile water or the like may also be used. A suitable buffer may also be present allowing the compositions to be lyophilized and stored in sterile conditions prior to reconstitution by the addition of sterile water for subsequent administration. Incorporation of the polypeptides of the invention into a solid or semi-solid biologically compatible matrix may be carried out which can be implanted into tissues requiring treatment.

The carrier can also contain other pharmaceutically acceptable excipients for modifying other conditions such as pH, osmolarity, viscosity, sterility, lipophilicity, solubility or the like. Pharmaceutically acceptable excipients which permit sustained or delayed release following administration may also be included.

The polypeptides, the nucleic acid molecules or compounds according to the invention may be administered orally. In this embodiment they may be encapsulated and combined with suitable carriers in solid dosage forms which would be well known to those skilled in the art.

As would be well known to those of skill in the art, the specific dosage regime may be calculated according to the body surface area of the patient or the volume of body space to be occupied, dependent upon the particular route of administration to be used. The amount of the composition actually administered will, however, be determined by a medical practitioner, based on the circumstances pertaining to the disorder to be treated, such as the severity of the symptoms, the composition to be administered, the age, weight, and response of the individual patient and the chosen route of administration.

**Example 1**

**Identification of novel drug targets in *C. albicans* by anti-sense and disruptive integration**

The principle of the approach is based on the fact that when a particular *C. albicans* mRNA is inhibited by producing the complementary anti-sense RNA, the corresponding protein will decrease. If this protein is critical for growth or survival, the cell producing the anti-sense RNA will grow more slowly or will die.

Since anti-sense inhibition occurs at mRNA level, the gene copy number is irrelevant, thus allowing applications of the strategy even in diploid organisms.

Anti-sense RNA is endogenously produced from an integrative or episomal plasmid with an inducible promoter; induction of the promoter leads to the production of an RNA encoded by the insert of the plasmid. This insert will differ from one plasmid to another in the library. The inserts will be derived from genomic DNA fragments or from cDNA to cover-to the extent possible- the entire genome.

The vector is a proprietary vector allowing

integration by homologous recombination at either the homologous insert or promoter sequence in the *Candida* genome. After introducing plasmids from cDNA or genomic libraries into *C. albicans*, transformants are screened for impaired growth after promoter (& thus anti-sense) induction in the presence of lithium acetate. Lithium acetate prolongs the G1 phase and thus allows anti-sense to act during a prolonged period of time during the cell cycle. Transformants which show impaired growth in both induced and non-induced media, thus showing a growth defect due to integrative disruption, are selected as well.

Transformants showing impaired growth are supposed to contain plasmids which produce anti-sense RNA to mRNAs critical for growth or survival. Growth is monitored by measuring growth-curves over a period of time in a device (Bioscreen Analyzer, Labsystems) which allows simultaneous measurement of growth-curves of 200 transformants.

Subsequently plasmids can be recovered from the transformants and the sequence of their inserts determined, thus revealing which mRNA they inhibit. In order to be able to recover the genomic or cDNA insert which has integrated into the *Candida* genome, genomic DNA is isolated, cut with an enzyme which cuts only once into the library vector (and estimated approx. every 4096 bp in the genome) and religated. PCR with primers flanking the insert will yield (partial) genomic or cDNA inserts as PCR fragments which can directly be sequenced. This PCR analysis (on ligation reaction) will also show us how many integrations occurred. Alternatively the ligation reaction is transformed to E. coli and PCR analysis is performed on colonies or on plasmid DNA derived thereof.

This method is employed for a genome-wide search for novel *C. albicans* genes which are important for growth or survival.

## Materials & Methods

### Construction of pGal1PNiST-1 (SEQ ID NO:21)

The backbone of the pGAL1PNiST-1 vector (integrative anti-sense *Sfi*I-*Not*I vector) is pGEM11Zf(+) (Promega Inc.). First, the CaMAL2 *Eco*RI/*Sal*I promoter fragment from pDBV50 (D.H. Brown *et al.* 1996) was ligated into *Eco*RI/*Sal*I-opened pGEM11Zf(+) resulting in the intermediate construct pGEMMAL2P-1. Into the latter (*Msc*I/CIP) the CaURA3 selection marker was cloned as a *Eco*47III/*Xmn*I fragment derived from pRM2. The resulting pGEMMAL2P-2 vector was *Not*I/*Hin*dIII opened in order to accept the *Not*I-stuffer-*Sfi*I cassette from pPCK1NiSCYCT-1 (*Eag*I/*Hin*dIII fragment): pMAL2PNiST-1. Finally, the plasmid pGAL1PNiST-1 was constructed by exchanging the *Sal*I/*Ecl*136II MAL2 promoter in pMAL2PNiST-1 by the *Xho*I/*Sma*I GAL1 promoter fragment derived from pRM2GAL1P.

### Construction of pGal1PSiST-1 (SEQ ID ON:22)

The vector pGAL1PSiST-1 was created for cloning the small genomic DNA fragments (flanked by *Sfi*I sites) behind the GAL1 promoter. The only difference with pGAL1PNiST-1 is that the hIFNβ (stuffer fragment) insert fragment in pGAL1PSiST-1 is flanked by two *Sfi*I sites in stead of a *Sfi*I and a *Not*I site as in pGAL1PNiST-1. To construct pGAL1PSiST-1 the *Eco*RI-*Hind*III fragment, containing hIFNβ flanked by a *Sfi*I and a *Not*I site, of pMAL2pHiET-3 (unpublished) was exchanged by the *Eco*RI-*Hind*III fragment, containing hIFNβ flanked by two *Sfi*I sites, from YCp50S-S (an *E. coli / S. cerevisiae* shuttle vector derived from the plasmid YCp50, which is deposited in the ATCC collection (number 37419; Thrash *et al.*, 1985); an

*Eco*RI-*Hind*III fragment, containing the gene hIFNβ, which is flanked by two *Sfi*I sites, was inserted in YCp50, creating YCp50S-S), resulting into plasmid pMAL2PSiST-1. The *MAL*2 promoter from pMAL2PSiST-1 (by a *Nae*I-*bal*I digest) was further replaced by the *GAL*1 promoter from pGAL1PNiST-1 (via a *Xho*I-*FSP*I digest), creating the vector pGAL1PSiST-1.

***Candida albicans* genomic library**

* *Preparation of the genomic DNA fragments*

A *Candida albicans* genomic DNA library with small DNA fragments (400 to 1,000 bp) was prepared. Genomic DNA of *Candida albicans* B2630 was isolated following a modified protocol of Blin and Stafford (1976). The quality of the isolated genomic DNA was checked by gel electrophoresis. Undigested DNA was located on the gel above the marker band of 26,282 bp. A little smear, caused by fragmentation of the DNA, was present.

To obtain enrichment for genomic DNA fragments of the desired size, the genomic DNA was partially digested. Several restriction enzymes (*Alu*I, *Hae*III and *Rsa*I; all creating blunt ends) were tried out. The appropriate digest conditions have been determined by titration of the enzyme. Enrichment of small DNA fragments was obtained with 70 units of *Alu*I on 10 μg of genomic DNA for 20 min. T4 DNA polymerase (Boehringer) and dNTPs (Boehringer) were added to polish the DNA ends. After extraction with phenol-chloroform the digest was size-fractionated on an agarose gel. The genomic DNA fragments with a length of 500 to 1,250 bp were eluted from the gel by centrifugal filtration (Zhu *et al.*, 1985). *Sfi*I adaptors (5' GTTGGCCTTTT) or (5' AGGCCAAC) were attached to the DNA ends (blunt) to facilitate cloning of the fragments into the vector. Therefore, a 8-mer and 11-mer oligonucleotide (comprising the *Sfi*I site)

were kinased and annealed. After ligation of these adaptors to the DNA fragments a second size-fractionation was performed on an agarose gel. The DNA fragments of 400 to 1150 bp were eluted from the gel by centrifugal filtration.

* *Preparation of the pGAL1PSiST-1 vector fragment*

The small genomic DNA fragments were cloned after the GAL1 promoter in the vector pGAL1PSIST-1. Qiagen-purified pGAL1PSiST-1 plasmid DNA was digested with *Sfi*I and the largest vector fragment eluted from the gel by centrifugal filtration (Zhu *et al.*, 1985). Ligation with a control DNA fragment, flanked by *Sfi*I sites, was performed as a control. The ligation mix was electroporated to MC1061 *E. coli* cells. Plasmid DNA of 24 clones was analyzed. In all cases the control fragment was inserted in the pGAL1PSiST-1 vector fragment.

* *Upscaling*

All genomic DNA fragments (450 ng) were ligated into the pGAL1PSiST-1 vector (20 ng). After electroporation at 2500V, 40µF circa 400,000 clones were obtained. These clones were pooled into three groups and stored as glycerol slants. Also Qiagen-purified DNA was prepared from these clones. A clone analysis showed an average insert length of 600 bp and a percentage of 91 for clones with an insert. The size of the library corresponds to 5 times the diploid genome. The genomic DNA inserts are sense or anti-sense orientated in the vector.

***Candida albicans* cDNA library**

Total RNA was extracted from *Candida albicans* B2630 grown on respectively minimal (SD) and rich (YPD) medium as described by Chirgwin *et al.* in Sambrook *et al* 1996. mRNA was prepared from total RNA using the Invitrogen Fast Track procedure.

First strand cDNA is synthesised with the

Superscript Reverse Transcriptase (BRL) and with an oligo dT-*Not*I Primer adapter. After second strand synthesis, cDNA is polished with Klenow enzyme and purified over a Sephacryl S-400 spun column. Phosphorylated *Sfi*I adapters are then ligated to the cDNA, followed by digestion with the *Not*I restriction enzyme. The *Sfi*I/*Not*I cDNA is then purified and sized on a Biogel column A150M.

First fraction contains approximately 38,720 clones by transformation, the second fraction only 1540 clones. Clone analysis:

Fr. I: 22/24 inserts, 16 ; 1000 bp, 4 ; 2000 bp, average size: 1500 bp.

Fr. II: 9/12 inserts, 3 ; 1000 bp, average size: 960 bp cDNA was ligated in a *Not*I/*Sfi*I opened pGAL1PNiST-1 vector (anti-sense)

**_Candida_ transformation**

The host strain used for transformation is a *C. albicans* ura3 mutant, CAI-4, which contains a deletion in orotidine-5=-phosphate decarboxylase and was obtained from William Fonzi, Georgetown University (Fonzi and Irwin). CAI-4 was transformed with the above described cDNA library or genomic library using the *Pichia* spheroplast module (Invitrogen). Resulting transformants were plated on minimal medium supplemented with glucose (SD, 0.67% or 1.34% Yeast Nitrogen base w/o amino acids + 2% glucose) plates and incubated for 2-3 days at 30°C.

**Screening for mutants**

Starter cultures were set up by inoculating each colony in 1 ml SD medium and incubating overnight at 30°C and 300 rpm. Cell densities were determined using a Coulter counter (Coulter Z1; Coulter electronics limited). 250.000 cells/ml were inoculated in 1 ml SD

medium and cultures were incubated for 24 hours at 30°C and 300 rpm. Cultures were washed in minimal medium without glucose (S) and the pellet resuspended in 650 μl S medium. 8 μl of this culture is used for inoculating 400 μl cultures in a Honeywell-100 plate (Bioscreen analyzer; Labsystems). Each transformant was grown during three days in S medium containing LiAc; pH 6.0, with 2% glucose/2% maltose or 2% galactose/2% maltose respectively while shaking every 3 minutes for 20 seconds. Optical densities were measured every hour during three consecutive days and growth curves were generated (Bioscreen analyzer; Labsystems).

Growth curves of transformants grown in respectively anti-sense non-inducing (glucose/maltose) and inducing (galactose/maltose) medium are compared and those transformants showing impaired growth upon anti-sense induction are selected for further analysis. Transformants showing impaired growth by virtue of integration into a critical gene are also selected.

**Isolation of genomic or cDNA inserts**

Putatively interesting transformants are grown in 1.5 ml SD overnight and genomic DNA is isolated using the Nucleon MI Yeast kit (Clontech). Concentration of genomic DNA is estimated by analyzing a sample on an agarose gel.

20 ng of genomic DNA is digested for three hours with an enzyme that cuts uniquely in the library vector (SacI for the genomic library; PstI for the cDNA library) and treated with RNAse. Samples are phenol/chloroform extracted and precipitated using NaOAc/ethanol.

The resulting pellet is resuspended in 500 μl ligation mixture (1 x ligation buffer and 4 units of

T4 DNA ligase; both from Boehringer) and incubated overnight at 16°C.

After denaturation (20 min 65°C), purification (phenol/chloroform extraction) and precipitation (NaOAc/ethanol) the pellet is resuspended in 10 $\mu$l MilliQ (Millipore) water.

**PCR analysis**

Inverse PCR is performed on 1 $\mu$l of the precipitated ligation reaction using library vector specific primers (oligo23 5' TGC-AGC-TCG-ACC-TCG-ACT-G 3' SEQ ID NO: 17 and oligo25 5' GCG-TGA-ATG-TAA-GCG-TGA-C 3' SEQ ID NO: 18 for the genomic library; 3pGALNistPCR primer: 5'TGAGCAGCTCGCCGTCGCGC 3' SEQ ID NO: 19 and 5pGALNistPCR primer: 5'GAGTTATACCCTGCAGCTCGAC 3' SEQ ID NO: 20 for the cDNA library; both from Eurogentec) for 30 cycles each consisting of (a) 1 min at 95 °C, (b) 1 min at 57 °C, and (c) 3 min at 72 °C. In the reaction mixture 2.5 units of Taq polymerase (Boehringer) with TaqStart antibody (Clontech) (1:1) were used, and the final concentrations were 0.2 $\mu$M of each primer, 3 mM MgCl2 (Perkin Elmer Cetus) and 200 $\mu$M dNTPs (Perkin Elmer Cetus). PCR was performed in a Robocycler (Stratagene).

**Sequence determination**

Resulting PCR products were purified using PCR purification kit (Qiagen) and were quantified by comparison of band intensity on EtBr stained agarose gel with the intensity of DNA marker bands. The amount of PCR product (expressed in ng) used in the sequencing reaction is calculated as the length of the PCR product in basepairs divided by 10. Sequencing reactions were performed using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit

according to the instructions of the manufacturer (PE Applied Biosystems, Foster City, CA) except for the following modifications.

The total reaction volume was reduced to 15 µl. Reaction volume of individual reagents were changed accordingly. 6.0 µl Terminator Ready Reaction Mix was replaced by a mixture of 3.0 µl Terminator Ready Reaction Mix + 3.0 µl Half Term (GENPAK Limited, Brighton, UK). After cycle sequencing, reaction mixtures were purified over Sephadex G50 columns prepared on Multiscreen HV opaque microtiter plates (Millipore, Molsheim, Fr) and were dried in a speedVac. Reaction products were resuspended in 3 µl loading buffer. Following denaturation for 2 min at 95°C, 1 µl of sample was applied on a 5% Long Ranger Gel (36 cm well-to-read) prepared from Singel Packs according to the supplier's instructions (FMC BioProducts, Rockland, ME). Samples were run for 7 hours 2X run on a ABI 377XL DNA sequencer. Data collection version 2.0 and Sequence analysis version 3.0 (for basecalling) software packages are from PE Applied Biosystems. Resulting sequence text files were copied onto a server for further analysis.

**Sequence analysis**

Nucleotide sequences were imported in the VectorNTI software package (InforMax Inc, North Bethesda, MD, USA), and the vector and insert regions of the sequences were identified. Sequence similarity searches against public and commercial sequence databases were performed with the BLAST software package (Altschul *et al.*, 1990) version 1.4. Both the original nucleotide sequence and the six-frame conceptual translations of the insert region were used as query sequences. The used public databases were the EMBL nucleotide sequence database (Stoesser *et al.*,

1998), the SWISS-PROT protein sequence database and its supplement TrEMBL (Bairoch and Apweiler, 1998), and the ALCES *Candida albicans* sequence database (Stanford University, University of Minnesota). The commercial sequence databases used were the LifeSeq® human and PathoSeq® microbial genomic databases (Incyte Pharmaceuticals Inc., Palo Alto, CA, USA), and the GENESEQ patent sequence database (Derwent, London, UK). Three major results were obtained on the basis of the sequence similarity searches: function, novelty, and specificity. A putative function was deduced on the basis of the similarity with sequences with a known function, the novelty was based on the absence or presence of the sequences in public databases, and the specificity was based on the similarity with vertebrate homologues.

**Methods**

Blastx of the nucleic acid sequences against the appropriate protein databases: Swiss-Prot for clones of which the complete sequence is present in the public domain, and paorfp (PathoSeq™) for clones of which the complete sequences is not present in the public domain.

The protein to which the translated nucleic acid sequence corresponds to is used as a starting point. The differences between this protein and our translated nucleic acid sequences are marked with a double line and annotated above the protein sequence. The following symbols are used:

A one-letter amino acid code or the ambiguity code X is used if our translated nucleic acid sequence has another amino acid on a certain position,

The stop codon sign * is used if our translated nucleic acid sequence has a stop codon on a certain position,

The letters fs (frame shift) are used if a frame

shift occurs in our translated nucleic acid sequence, and another reading frame is used,

The words ambiguity or ambiguities are used if a part of our translated nucleic acid sequence is present in the proteins, but not visible in the alignments of the blast results,

The phrase "missing sequence" is used if the translated nucleic acid sequence does not comprise that part of the protein.

Blastx: compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database.

**Antisense Experiments**

Clones 383c_cp, 392c_cp, 417c_cpG2L, 325c_af, 322c_cp, 26g3, and 409c_cp were transformed with plasmid pGAL1PSiST-1 containing the galactose inducible promotor/expression cassette. The plasmid was modified to include antisense molecules capable of binding to RNA expressed from DNA corresponding to SEQ ID NOs: 1-2 and SEQ ID NOs 4-8 natively present the *Candida albicans* cell. Following transformation the cells were grown in the presence of glucose or galactose to determine the effect of the induced antisense molecule on protein expression and on *Candida albicans* cell growth. An exemplary growth curve is provided in Figure 5. All cells transformed with the antisense molecules demonstrated significant growth reduction.

**Gene Knock-outs**

To verify that the growth effect was due to the interference with the identified gene and to support the specificity of the antisense effect, single and double allele knock-outs were made in the identified genes using the URA-blaster method (Fonzi and Irwin

1993). Figure 4 provides an example of a growth curve for a double allele knock-out as compared to the wild-type strain CAI4. Growth curves are shown as the measurement of optical density (Y-axis) over time in hours (X-axis). The optical density is a measure for the number of cells present and this a measure for growth. A reduction in growth was observed for this double knock-out as compared to the wild-type strain.

**Screening for compounds modulating expression of polypeptides critical for growth and survival of *C. albicans***

The method proposed is based on observations (Sandbaken *et al.*, 1990; Hinnebusch and Liebman 1991; Ribogene PCT WO 95/11969, 1995) suggesting that underexpression or overexpression of any component of a process (e.g. translation) could lead to altered sensitivity to an inhibitor of a relevant step in that process. Such an inhibitor should be more potent against a cell limited by a deficiency in the macromolecule catalyzing that step and/or less potent against a cell containing an excess of that macromolecule, as compared to the wild type (WT) cell.

Mutant yeast strains, for example, have shown that some steps of translation are sensitive to the stoichiometry of macromolecules involved. (Sandbaken *et al.* 1996). Such strains are more sensitive to compounds, which specifically perturb translation (by acting on a component that participates in translation) but are equally sensitive to compounds with other mechanisms of action.

This method thus not only provides a means to identify whether a test compound perturbs a certain process but also an indication of the site at which it exerts its effect. The component which is present in altered form or amount in a cell whose growth is

affected by a test compound is potentially the site of action of the test compound.

The assay involves measurement of growth of an isogenic strain which has been modified only in a certain specific allele, relative to a wild type (WT) *C. albicans* strain, in the presence of R-compounds. Strains can be ones in which the expression of a specific essential protein is impaired upon induction of anti-sense or strains which carry disruptions in an essential gene. An *in silico* approach to finding novel essential genes in *C. albicans* will be performed. A number of essential genes identified in this way will be disrupted (in one allele) and the resulting strains can be used for comparative growth screening.

**Assay for High Throughput screening for drugs**

35 µl minimal medium (S medium + 2% galactose + 2% maltose) is transferred in a transparent flat-bottomed 96 well plate using an automated pipetting system (Multidrop, Labsystems). A 96-channel pipettor (Hydra, Robbins Scientific) transfers 2.5 µl of R-compound at $10^{-3}$ M in DMSO from a stock plate into the assay plate.

The selected C. *albicans* strains (mutant and parent (CAI-4) strain) are stored as glycerol stocks (15%) at -70°C. The strains are streaked out on selective plates (SD medium) and incubated for two days at 30°C. For the parent strain, CAI-4, the medium is always supplemented with 20 µg/ml uridine. A single colony is scooped up and resuspended in 1 ml minimal medium (S medium + 2% galactose + 2% maltose). Cells are incubated at 30°C for 8 hours while shaking at 250 rpm. A 10 ml culture is inoculated at 250,000 cells/ml. Cultures are incubated at 30°C for 24 hours while shaking at 250 rpm. Cells are counted in Coulter counter and the final culture (S medium + 2%

galactose + 2% maltose) is inoculated at 20,000 to 50,000 cells/ml. Cultures are grown at 30°C while shaking at 250 rpm until a final OD of 0.24 (+/- 0.04) 600nM is reached.

200 µl of this yeast suspension is added to all wells of MW96 plates containing R-compounds in a 450 (or 250) µl total volume. MW96 plates are incubated (static) at 30°C for 48 hours.

Optical densities are measured after 48 hours.

Test growth is expressed as a percentage of positive control growth for both mutant (x) and wild type (y) strains. The ratio (x/y) of these derived variables is calculated.

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**Table 1**

| Seq ID No. | Clone | Function |
|---|---|---|
| 1 | 382c_cp | - |
| 2 | 392c_cp | TUF1 |

| | | |
|---|---|---|
| 3 | - | RAD53 |
| 4 | 417c_cpG2L | - |
| 5 | 325c_af | - |
| 6 | 322c_cp[1] | - |
| 7 | 26g3 | - |
| 8 | 409c_cp | - |
| 9 | 382c_cpG1L2 | - |
| 10 | 382c_cp (prt) | - |
| 11 | 392c_cp (prt) | TUF1 |
| 12 | | RAD53 |
| 13 | 325c_af (prt)[2] | - |
| 14 | 322c_cp (prt)[2] | - |
| 15 | 26g3 (prt) | - |
| 16 | 417c_cp 92L (prt) | - |

1. 322c-cp is a member of the UPF0057 protein family. It contains potential transmembrane regions (6-23aa; 30-53aa) and could be low temperature or salt-stress inducible.

2. 325c-af shows similarity to IMP4 yeast and related proteins and it might be involved in rRNA processing in *Candida albicans* in a similar way to IMP4.

**What is claimed is:**

1. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

2. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 3.

3. A nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from SEQ. ID NO: 1 or SEQ. ID. NO: 2 and fragments or derivatives of said nucleic acid molecules.

4. The nucleic acid molecule of Claim 1 which is mRNA.

5. The nucleic acid molecule of Claim 1 which is DNA.

6. The nucleic acid molecule according to claim 5 which is cDNA.

7. A first nucleic acid molecule capable of hybridising to a second nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which second nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 wherein hybridsation occurs under high stringency conditions.

8. An antisense molecule comprising a nucleic acid

molecule capable of hybridising to a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* wherein the nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

9. Cells containing a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein said cells are bacterial or eukaryotic.

10. A polypeptide encoded by a nucleic acid molecule which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

11. A polypetide having an amino acid sequence selected from the group consisting of SEQ ID NO: 10 to SEQ ID NO: 16.

12. A recombinant DNA construct comprising a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein the nucleic acid molecule is DNA.

13. A recombinant DNA construct comprising a first nucleic acid molecule wherein the first nucleic acid molecule or a molecule complementary to the first nucleic acid molecule is capable of hybridizing a second nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which second nucleic acid

molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 wherein the first nucleic acid molecule is inserted in the antisense orientation.

14. A recombinant DNA construct according to claim 12 wherein said recombinant DNA construct is an expression vector.

15. The construct according to claim 14 which comprises an inducible promoter.

16. The construct according to claim 14 which comprises a sequence encoding a reporter molecule.

17. Cells containing a recombinant DNA construct comprising a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein said cells are bacterial or eukaryotic.

18. The nucleic acid molecule according to claim 1 in a pharmaceutically acceptable carrier, diluent, excipient or buffer.

19. A method for treating a *Candida albicans*-associated disease comprising the step of: administering a composition of matter comprising an antisense nucleic acid molecule capable of binding to a *Candida albicans*-originating nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9.

20. A pharmaceutical composition comprising a polypeptide according to claim 10 together with a

pharmaceutically acceptable carrier diluent or excipient therefor.

21. A *Candida albicans* cell comprising an induced mutation in the DNA sequence encoding the polypeptide according to claim 10.

22. A method of identifying compounds which selectively modulate expression or functionality of polypeptides or metabolic pathways in which these polypeptides are involved and which are crucial for growth and survival of *Candida albicans*, which method comprises:

(a) contacting a compound to be tested with one or more *Candida albicans* cells having a mutation in a nucleic acid molecule according to claim 1 which mutation results in overexpression or underexpression of said polypeptides in addition to contacting one or more wild type *Candida albicans* cells with said compound; and

(b) monitoring the growth and/or activity of said mutated cell compared to said wild type; wherein differential growth or activity of said one or more mutated *Candida* cells is indicative of selective action of said compound on a polypeptide or another polypeptide in the same or a parallel pathway.

23. A compound identifiable according to the method of claim 22.

24. A pharmaceutical composition comprising a compound according to claim 23 together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

25. A method of identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are critical for growth or survival of said cell or organism, which method comprises:

(a) preparing a cDNA or genomic library from said cell or organism in a suitable expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription of antisense RNA from the nucleotide sequences in said cDNA or genomic library; and

(b) selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant.

26. The method according to claim 25 wherein said cell or organism is a yeast or filamentous fungus.

27. The method according to claim 25 wherein said cell or organism is selected from the group consisting of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

28. An antibody capable of binding to a polypeptide encoded by a nucleic acid molecule which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

29. An oligonucleotide comprising a fragment of from 10 to 120 contiguous nucleotides of a nucleic acid molecule which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid

molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

30. The oligonucleotide according to claim 29 comprising a fragment of from 10 to 50 contiguous nucleotides.

## ABSTRACT

### DRUG TARGETS IN *CANDIDA ALBICANS*

The present invention is concerned with the identification of genes or functional fragments thereof from *Candida albicans* which are critical for growth and cell division and which genes may be used as selective drug targets to treat *Candida albicans* associated infections. Novel nucleic acid sequences from *Candida albicans* are also provided and which encode the polypeptides which are critical for growth of *Candida albicans*. Methods for the identification of anti-fungal compounds which inhibit fungal or yeast growth are also contemplated.

09/857372

# FIGURE 1

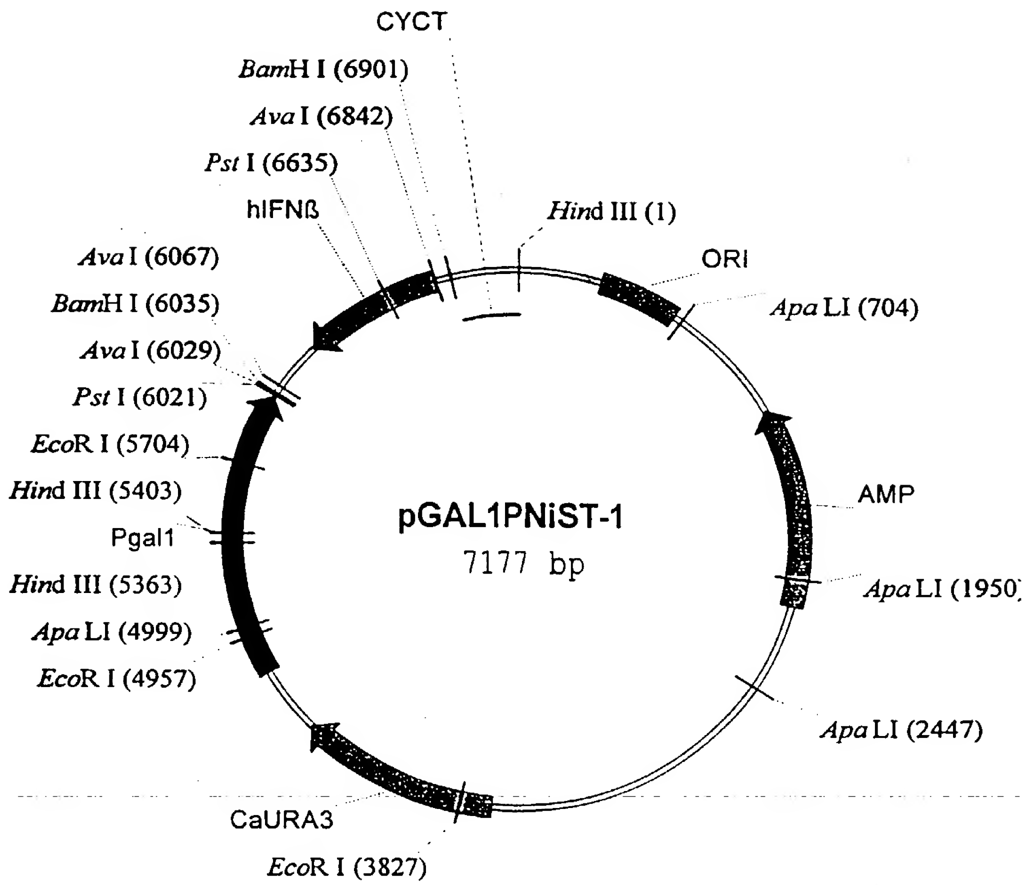

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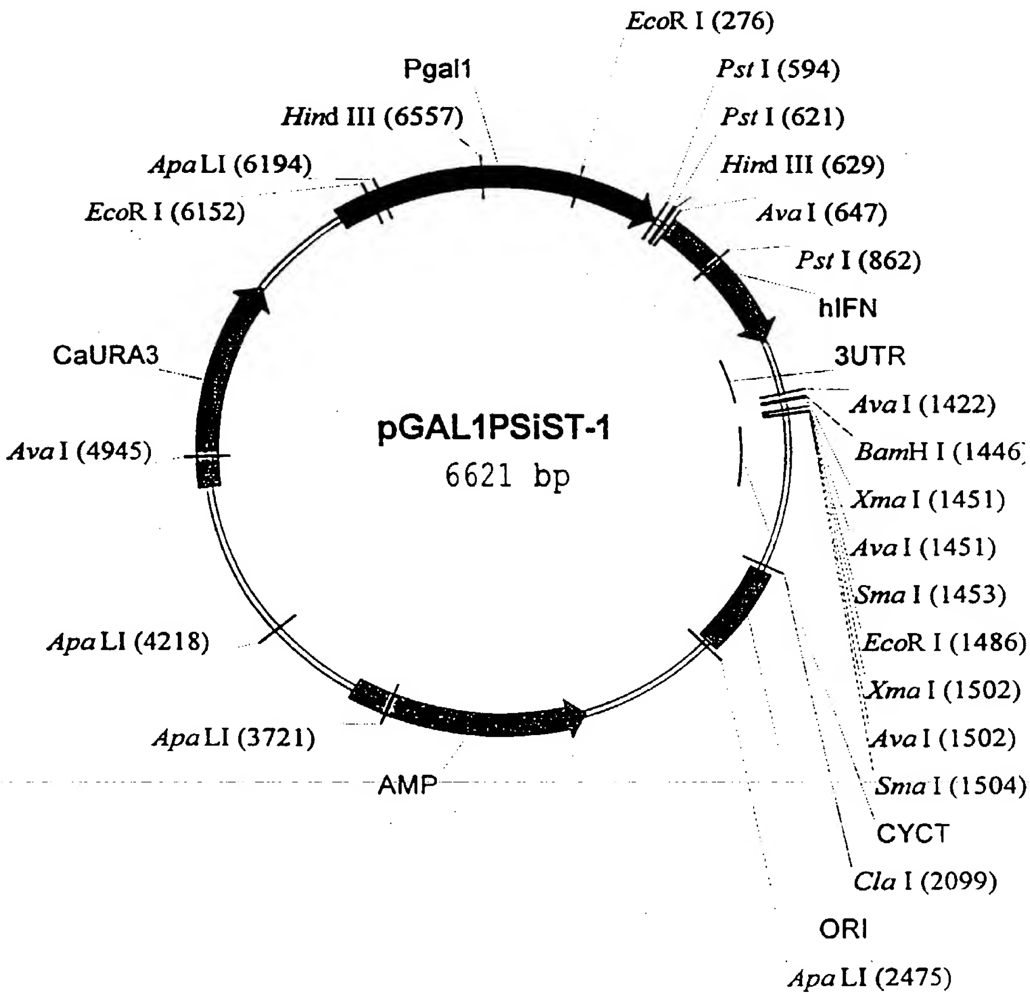
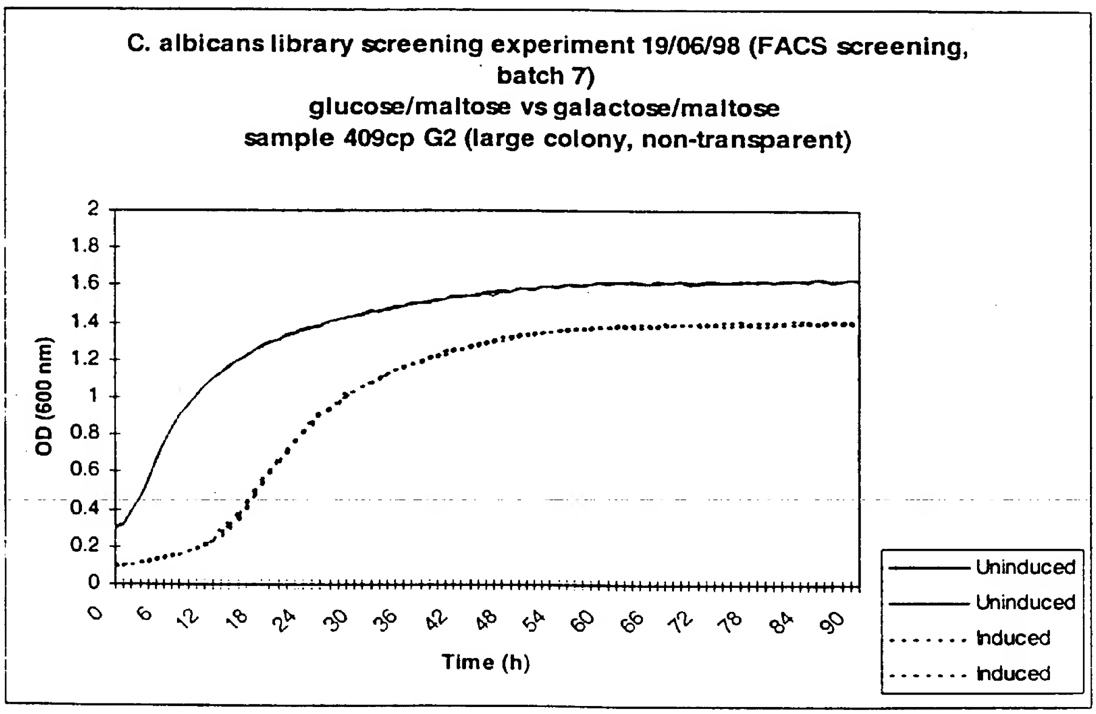
FIGURE 2

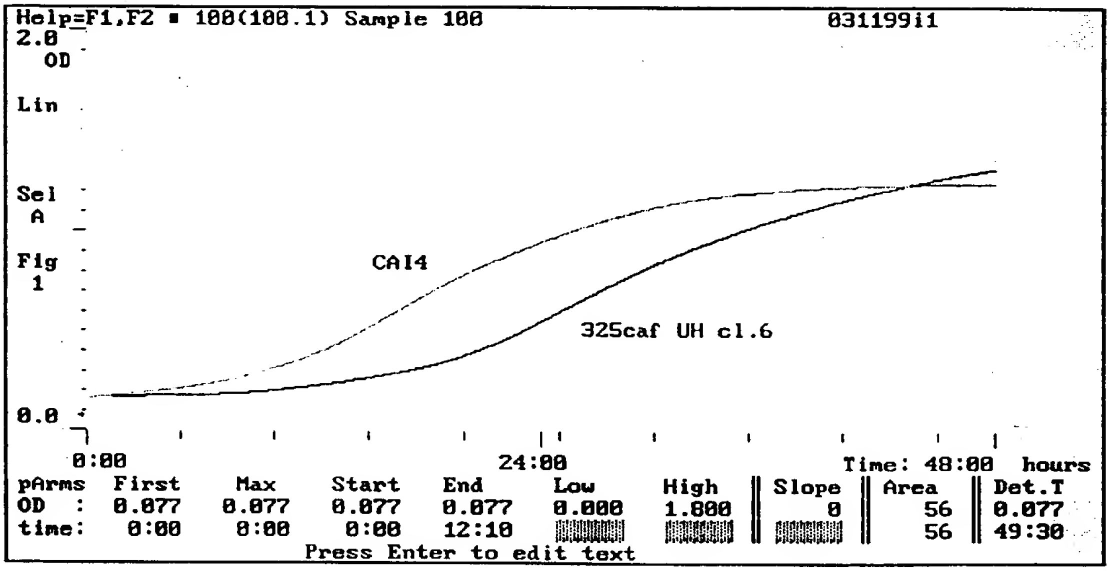


# FIGURE 3


C. albicans library screening experiment 19/06/98 (FACS screening, batch 7)
glucose/maltose vs galactose/maltose
sample 409cp G2 (large colony, non-transparent)
OD (600 nm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
Time (h)
Uninduced
Uninduced
Induced
Induced

09/857372

# FIGURE 4



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# DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☒ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

| | |
|---|---|
| Attorney Docket Number | JAB 1430-PCT-USA |
| First Named Inventor | Contreras, Roland Henri |
| ***COMPLETE IF KNOWN*** | |
| Application Number | / |
| Filing Date | |
| Group Art Unit | |
| Examiner Name | |

**As a below named inventor, I hereby declare that:**

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DRUG TARGETS IN CANDIDA ALBICANS

*(Title of the Invention)*

the specification of which

☐ is attached hereto

OR

☐ was filed on (MM/DD/YYYY) 12/06/1999 as United States Application Number or PCT International Application Number PCT/EP99/09833 and was amended on (MM/DD/YYYY) ____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

| Prior Foreign Application Number(s) | Country | Foreign Filing Date (MM/DD/YYYY) | Priority Not Claimed | Certified Copy Attached? YES | NO |
|---|---|---|---|---|---|
| 98204122.0 | EP | 12/04/1998 | ☐ | ☐ | ☐ |
| | | | ☐ | ☐ | ☐ |
| | | | ☐ | ☐ | ☐ |
| | | | ☐ | ☐ | ☐ |

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

| Application Number(s) | Filing Date (MM/DD/YYYY) |
|---|---|
| | |

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I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

| U.S. Parent Application or PCT Parent Number | Parent Filing Date (MM/DD/YYYY) | Parent Patent Number *(if applicable)* |
|---|---|---|
| | | |

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*OR*
☐ Registered practitioner(s) name/registration number listed below

| Name | Registration Number | Name | Registration Number |
|---|---|---|---|
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| Steven P. Berman | 24,772 | Ellen C. Coletti | 34,140 |
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**Name of Sole or First Inventor:** ☐ A petition has been filed for this unsigned inventor

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☐ Additional inventors are being named on the ____ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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2

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| Jorge Eduardo | | Vialard | | | |
| Inventor's Signature | Jorge Vialard | | | Date | 03/20/01 |
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| | | | | | | | |
|---|---|---|---|---|---|---|---|
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| Given Name (first and middle [if any]) | Marianne Denise | Family Name or Surname | De Backer | | | | |
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| **Name of Additional Joint Inventor, if any:** | | ☐ A petition has been filed for this unsigned inventor | | | | | |
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| Post Office Address | | | | | | | |
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| **Name of Additional Joint Inventor, if any:** | | ☐ A petition has been filed for this unsigned inventor | | | | | |
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| Given Name (first and middle [if any]) | Marc George | Family Name or Surname | Logghe | | | | |
| Inventor's Signature | | | | | Date | 03/22/01 | |
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| Post Office Address | | | | | | | |
| City | St.-Denijs-Westrem | State | | ZIP | 9051 | Country | Belgium |

| **Name of Additional Joint Inventor, if any:** | | ☐ A petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name (first and middle [if any]) | Jorge Eduardo | Family Name or Surname | Vialard | | | | |
| Inventor's Signature | | | | | Date | | |
| Residence: City | Beerse | State | | Country | Belgium | Citizenship | CA |
| Post Office Address | c/o Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium | | | | | | |
| Post Office Address | | | | | | | |
| City | Brussel | State | | ZIP | 1200 | Country | Belgium |

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DOCKET NO. JAB-1430

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : CONTRERAS et al.

Serial No. : Art Unit:

Filed : June 4, 2001 Examiner:

For : DRUG TARGETS IN CANDIDA ALBICANS

Commissioner for Patents
Washington, D.C. 20231

ASSOCIATE POWER OF ATTORNEY

Sir:

In the matter of the above-identified application, I hereby appoint Myra H. McCormack (Reg. No. 36,602), whose postal address is One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933-7003, my associate attorney to prosecute said application, to make alterations and amendments therein, to file continuing applications claiming the benefit of said application, to receive the patent and to transact all business in the Patent Office connected with said application.

I request all communications with respect to said application be addressed to Philip S. Johnson, One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933-7003. All telephone calls should be directed to Myra H. McCormack (732) 524-6932.

Signed at New Brunswick, in the County of Middlesex and State of New Jersey, this 4th day of June, 2001.

Mary A. Appollina

Mary A. Appollina
Reg. No. 34,087
Attorney for Applicant(s)

One Johnson & Johnson Plaza
New Brunswick, NJ 08933-7003
(732) 524-3742
DATED: June 4, 2001

SEQUENCE LISTING

<110> Contreras, Roland
Nelissen, Bart
DeBacker, Marianne
Luyten, Walter
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Met Glu Ser Gln Gln Lys Asp Asn Tyr Ser Ser Leu Val Asp Ile Trp
        355                 360                 365

Ser Leu Gly Cys Leu Val Tyr Val Leu Leu Thr Ser His Leu Pro Phe
    370                 375                 380

Asn Gly Lys Asn Gln Gln Gln Met Phe Ala Lys Ile Lys Arg Gly Glu
385                 390                 395                 400

Phe His Glu Ala Pro Leu Asn Ser Tyr Asp Ile Ser Glu Asp Gly Arg
                405                 410                 415

Asp Phe Leu Gln Cys Cys Leu Gln Val Asn Pro Lys Leu Arg Met Thr
            420                 425                 430

Ala Ala Glu Ala Leu Lys His Lys Trp Leu Gln Asp Leu Tyr Glu Glu
        435                 440                 445

Asp Ser Val Lys Ser Leu Ser Leu Ser Gln Ser Gln Ser Gln Gln Ser
    450                 455                 460

Arg Lys Ile Asp Asn Gly Ile His Ile Glu Ser Leu Ser Lys Ile Asp
465                 470                 475                 480

Glu Asp Val Met Leu Arg Pro Leu Asp Ser Glu Arg Asn Arg Lys Ser
                485                 490                 495

Ser Lys Gln Gln Asp Phe Lys Val Pro Lys Arg Val Ile Pro Leu Ser
            500                 505                 510

Gln His Pro Ala Thr Pro Leu Pro Met Ser Gln Pro Lys Lys Arg Pro
        515                 520                 525

Tyr Gln Ile Asp Pro Arg Thr Asn Lys Lys Val Asp Leu Glu Glu Pro
    530                 535                 540

Ser Thr Ser Lys Lys Val Lys Leu Ser Asp Ser Val Val Ala Glu Asp
545                 550                 555                 560
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Tyr Leu Lys Leu Gly Pro Leu Ala Asn Ser Leu Phe Gln Glu Thr Ile
                565                 570                 575

Asn Ile Ser Lys Ser Pro Phe Ser Phe Gly Arg Asn Asp Thr Cys Asp
            580                 585                 590

Cys Glu Ile Asp Asp Asp Arg Leu Ser Lys Leu His Cys Val Ile Thr
        595                 600                 605

Lys Glu Asn Asp Ser Ile Trp Leu Leu Asp Lys Ser Thr Asn Ser Cys
    610                 615                 620

Leu Val Asn Asn Thr Ser Val Gly Lys Gly Asn Lys Val Leu Leu Arg
625                 630                 635                 640

Gly Gly Glu Ile Leu His Leu Phe Phe Asp Pro Leu Ser Ser Gln His
                645                 650                 655

Ile Gly Phe Lys Val Val Leu Val Asp Gln Ser Ser Gly Glu His Lys
            660                 665                 670

Ser Gln Val Glu Val Leu Lys Gln Thr Ser Glu Glu Met Asn Ile Ile
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Pro Leu Ile Ser Gly Leu Ser Ser Ile Ser Ser
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<211> 295
<212> PRT
<213> Candida albicans

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Arg Arg Gln Lys Val Phe Ala Glu Ile Lys His Glu Lys Asn Lys Gln
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Arg His Lys Gln Arg Ala Glu Arg Ala Lys Glu Glu Arg Glu Asn Pro
        35                  40                  45

Glu Leu Arg Glu Glu Arg Ile Ala Ala Asn Ile Pro Asp Thr Ile Asp
    50                  55                  60

Ser Lys Arg Ile Tyr Asp Glu Thr Ile Ala Ala Glu Val Glu Gly Asp
65                  70                  75                  80

Asp Glu Phe Gln Ser Tyr Phe Thr Asn Leu Leu Glu Glu Pro Lys Ile
                85                  90                  95

Leu Leu Thr Thr Ser Ala Asn Ala Lys Lys Pro Ala Tyr Glu Phe Ala
            100                 105                 110

Asp Met Ile Met Asp Phe Leu Pro Asn Val Thr Phe Ile Lys Arg Lys
        115                 120                 125

Lys Glu Tyr Thr Met Gln Asp Met Ala Lys Tyr Cys Ser Asn Arg Asp
    130                 135                 140
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Phe Thr Ala Leu Leu Val Ile Asn Glu Asp Lys Lys Lys Val Asn Gly
145 150 155 160

Ile Thr Leu Ile Asn Leu Pro Glu Gly Pro Thr Phe Tyr Phe Ser Ile
165 170 175

Thr Ser Ile Val Asp Gly Lys Arg Ile Lys Gly His Gly Lys Ala Gly
180 185 190

Asp Tyr Leu Pro Glu Ile Val Leu Asn Asn Phe Asn Ser Arg Leu Gly
195 200 205

Lys Thr Val Gly Arg Leu Phe Gln Ser Ile Phe Pro His Lys Pro Glu
210 215 220

Leu Gln Gly Arg Gln Val Ile Thr Leu His Asn Gln Arg Asp Tyr Ile
225 230 235 240

Phe Phe Arg Arg His Arg Tyr Ile Phe Arg Asn Glu Glu Lys Val Gly
245 250 255

Leu Gln Glu Gly Pro Gln Phe Thr Leu Lys Leu Arg Arg Met Gln Lys
260 265 270

Gly Val Arg Gly Asp Val Val Trp Glu His Arg Pro Asp Met Glu Arg
275 280 285

Asp Lys Lys Lys Phe Tyr Leu
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<211> 55
<212> PRT
<213> Candida albicans

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Pro Val Ala Val Phe Met Lys Cys Gly Ala Thr Thr Pro Leu Trp Ile
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Asn Leu Val Leu Cys Ile Phe Ile Trp Phe Pro Ala Ile Leu His Ala
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Leu Tyr Val Val Leu Lys Asp
50 55

<210> 15
<211> 773
<212> PRT
<213> Candida albicans

<400> 15
Met Thr Leu Gly Phe Asp Lys Phe Ile Ser Lys Val Ser Thr His Arg
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Arg Gln Ser Glu Pro Ser Ile Leu Glu Ile Ala Ala Thr Asn Ser Gln
20 25 30

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Asn Lys Ser Arg Arg Leu Ser Met Asp Asn Gly His Cys Tyr Val Arg
        35                  40                  45

Glu Ser Thr Asn Asn His His His Leu Asn Thr Val Val Asp Asn Leu
    50                  55                  60

Arg Gln Arg Ala Gly Ser Phe Ser Phe Ile Ser His His His Asn His
65                  70                  75                  80

His Gln Asn Ser His Asp Asn Tyr Thr Val Asp Pro Leu Thr Ser Asn
                85                  90                  95

Gly Ala Arg Ile Ser Arg Ser Arg Ser Arg Ser Lys Ser Val Gly His
            100                 105                 110

Gly Glu Ala Ile Ser Pro Ala Tyr Phe Ser Lys Asn Lys Thr Lys Asp
        115                 120                 125

Leu Val Lys Gln Glu Thr Ala His Ile Ile Ser Lys Lys Leu Leu Asn
    130                 135                 140

Met Leu Gln Asp Leu Asp Leu Gln Asn Pro Ile Ala Leu Lys Thr Ile
145                 150                 155                 160

Ser Gln Gly Ser Glu Ser Lys Phe Cys Lys Ile Tyr Val Ser Asn Thr
                165                 170                 175

Asn Asn Cys Ile Tyr Leu Pro Ala Ala Ser Ser Thr Ser Phe Thr Tyr
            180                 185                 190

Glu Asp Asp Glu Asn Gly Gly Val Ile Ile Ala Glu Asp Arg Asn Asp
        195                 200                 205

Glu Met Pro Thr Ala Val Asn Asn Asn Thr Leu Ser Met Asp Ser Ile
    210                 215                 220

Asn His Ser Glu Thr Asp Phe Ser Asp Ser Pro Pro Pro Pro Asp Leu
225                 230                 235                 240

Phe Ser Lys Met Lys Ser Phe His Ser Pro Asn Tyr Leu Thr Ser Lys
                245                 250                 255

Ile Asp Ser Glu Cys Pro Ile Pro His Thr Phe Ala Val Ile Val Glu
            260                 265                 270

Leu Thr Lys Asp Ser Leu Ile Ile Lys Asp Leu His Phe Gln Phe Gln
        275                 280                 285

Ser Leu Thr Thr Ile Leu Trp Pro Thr Gly Asp Ala Tyr Asn Arg Thr
    290                 295                 300

His Ala Lys Glu Lys Phe Thr Ile Gly Asn Met Glu Trp Arg Thr Ser
305                 310                 315                 320

Leu Ser Asp Ala Asp Tyr Tyr Ile Asn Ser Ser Asn Ser Asn Asp Val
                325                 330                 335

Lys Ser Lys Asn Leu Gly Pro Glu Asp Leu Ile Asn Arg Thr Arg Glu
            340                 345                 350

Tyr Lys Leu Ile Asp Ile Glu Glu Pro Asn Asn Ser Ser Asn Ser Leu
        355                 360                 365
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Ser Asp Asp Asp Met Asp Ile Asn Asn Ile Thr Ser Pro Leu Ser Thr
    370                 375                 380

Ser Pro Thr Ser Ser Ser Thr Ser Thr Asn Ser Thr Ser Asn Ser Leu
385                 390                 395                 400

Gly Ser Asp Ser Tyr Lys Ala Gly Leu Tyr Val Phe Leu Leu Pro Ile
                405                 410                 415

Leu Leu Pro Glu His Ile Pro Ala Ser Ile Val Ser Ile Asn Gly Ser
            420                 425                 430

Leu Ala His Thr Leu Ser Val Glu Cys Asn Lys Tyr Thr Asp Lys Leu
        435                 440                 445

Asn Arg Lys Ser Lys Val Ser Ala Ser Tyr Asn Leu Pro Met Val Arg
    450                 455                 460

Thr Pro Pro Asn Ile Gly Asn Ser Ile Ala Asp Lys Pro Ile Tyr Val
465                 470                 475                 480

Asn Arg Ile Trp Asn Asp Ala Val His Tyr Ile Ile Thr Phe Pro Arg
                485                 490                 495

Lys Tyr Val Thr Leu Gly Cys Glu His Met Ile Asn Val Lys Leu Ser
            500                 505                 510

Pro Met Val Lys Asp Val Val Ile Lys Arg Ile Lys Phe Asn Val Leu
        515                 520                 525

Glu Arg Ile Thr Tyr Val Ser Lys Asn Leu Ser Arg Glu Tyr Asp Tyr
    530                 535                 540

Asp Ser Glu Asp Pro Tyr Cys Ile His Pro Val Ser Lys Glu Asn Lys
545                 550                 555                 560

Val Arg Glu Arg Val Val Ser Leu Tyr Glu Leu Lys Thr Lys Ala Lys
                565                 570                 575

Gln Ser Ser Gly Gly His Leu Glu Ala Tyr Lys Gln Glu Val Met Lys
            580                 585                 590

Cys Pro Glu Asn Asn Leu Leu Phe Ser Cys Tyr Glu Val Glu Asn Asp
        595                 600                 605

Asn Asn Asn Gly Asn Gly Asn Gly Asn Gly Asn Gly Asn Lys Asn Val
    610                 615                 620

Lys Gln Lys Asn Lys Asp Gln Pro Met Ile Ala Thr Pro Leu Asp Ile
625                 630                 635                 640

Asn Val Ser Leu Pro Phe Leu Thr Thr Met Ser Asp Ser Leu Ile Met
                645                 650                 655

Thr Ser Ala Ile Glu Glu Glu Gly Ser Asp Ser Pro His Thr Ser Arg
            660                 665                 670

Arg Gly Ser Ala Val Ser Met Thr Asp Asn Asn Thr Thr Pro Ser Asn
        675                 680                 685

Asn Asn Pro Leu Ser Pro Phe Leu Gly Ala Val Glu Thr Asn Gly Ala
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690 695 700

Ser Ile Asn Glu Ile Gly Asp His Thr Leu Phe Pro Asp Ser Asn Phe
705 710 715 720

Arg His Ile Glu Ile Lys His Arg Leu Gln Val Thr Phe Arg Ile Ser
725 730 735

Lys Pro Asp Ser Asp Asn Lys Met His His Tyr Glu Val Val Ile Asp
740 745 750

Thr Pro Ile Val Leu Leu Ser Ser Lys Cys Gln Glu Asp Ser Pro Pro
755 760 765

Pro Tyr Ser Ser Val
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<210> 16
<211> 90
<212> PRT
<213> Candida albicans

<400> 16
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Thr Leu Cys Asn Arg Cys Gly Arg Arg Ser Phe His Val Gln Lys Lys
20 25 30

Thr Cys Ser Ser Cys Gly Tyr Pro Ala Ala Lys Met Arg Ser His Asn
35 40 45

Trp Ala Leu Lys Ala Lys Arg Arg Arg Thr Thr Gly Thr Gly Arg Met
50 55 60

Ala Tyr Leu Lys His Val Thr Arg Arg Phe Lys Asn Gly Phe Gln Thr
65 70 75 80

Gly Val Ala Lys Ala Gln Thr Pro Ser Ala
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<213> artificial sequence

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<223> Description of Artificial Sequence:primer

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<210> 18
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<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 18
gcgtgaatgt aagcgtgac 19

<210> 19
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<223> Description of Artificial Sequence:primer

<400> 19
tgagcagctc gccgtcgcgc 20

<210> 20
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<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 20
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<210> 21
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<212> DNA
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<223> Description of Artificial Sequence:DNA plasmid

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<210> 22
<211> 7127
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:DNA plasmid

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